Analytical method for pyrifluquinazon and its metabolites IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 in water

Reports:	ECM: EPA MRID No.: 50306301. Harper, H. 2013. Final Report - PYRIFLUQUINAZON: Validation of Methodology for the Determination of Residues of Pyrifluquinazon and Metabolites (IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203) in Ground, Surface and Drinking Water. Huntingdon Life Sciences Laboratory ID LMS/0075. Report prepared by Huntingdon Life Sciences, Suffolk, England, sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 136 pages (including pages 4B-4G). Final report issued January 24, 2013. Amendment 1 dated February 14, 2013 (p. 4). ILV: EPA MRID No. 49928711. Grant, J. 2013. PYRIFLUQUINAZON AND METABOLITES - INDEPENDENT LABORATORY VALIDATION OF HUNTINGDON LIFE SCIENCES' ANLAYTICAL PROCEDURE LMS/0075-01R "ANALYTICAL METHOD FOR THE DETERMINATION OF PYRIFLUQUINAZON AND METBAOLITES IN WATER". ABC Laboratories Study No. 69535. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 148 pages. Final report issued June 6, 2013.
Document No.:	MRIDs 50306301 & 49928711
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160), OECD, and European Good Laboratory Practices (GLP), as well as the Department of Health of the Government of the United Kingdom (pp. 3, 4F; Appendix 2, p. 129). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4, 4F-4G; Appendix 2, p. 129). A statement of the authenticity of the study report was not included. A statement of the authenticity of the study report amendment and reasons for the amendment was included (p. 4). ILV: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160) and OECD GLP standards (p. 3). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).
Classification:	This analytical method is classified as unacceptable. The reproducibility of the ECM method could not be determined since the validated LOQ of the ECM was not equivalent to the validated LOQ of the ILV for pyrifluquinazon and its metabolites. In the ILV, insufficient data was provided to support the linearity of the method for pyrifluquinazon and the precision and accuracy of the method for IV-203 in surface and drinking water. ECM matrices were not characterized. The LOD for the method differed between the ECM and ILV.
PC Code:	555555

EFED Final Reviewer:	James Lin Environmental Engineer	Signature: Date: 12/06/2018
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Date: 08/30/2018
	Joan Gaidos, Ph.D., Environmental Scientist	Signature: Date: 09/14/2018

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Huntingdon Life Sciences Analytical Procedure LMS/0075, is designed for the quantitative determination of pyrifluquinazon and its metabolites IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 at the LOQ of 0.10 µg/L in water matrices using LC/MS/MS. The LOQ is equal to the lowest toxicological level of concern in water for pyrifluquinazon, greater than the lowest toxicological level of concern in water for IV-01 and IV-02, and less than the lowest toxicological level of concern in water for IV-15, IV-27, IV-28 and IV-203. The reproducibility of the ECM method could not be determined since the validated LOQ of the ECM (0.1 µg/L) was not equivalent to the validated LOQ of the ILV (0.50 µg/L). Also, no ILV samples were prepared at the validated LOQ of the ECM (0.1 µg/L). The ILV validated the method in the first trial using characterized ground, surface and drinking water matrices with insignificant modifications to standard preparation and analytical procedure. In the ILV, insufficient data was provided to support the linearity of the method for pyrifluquinazon and the precision and accuracy of the method for IV-203 in surface and drinking water. All other ILV data regarding precision, accuracy, linearity, and specificity was acceptable for all analytes/matrices; however, only results and chromatograms from the quantitation ion transition were reported. All ECM data regarding precision, accuracy, linearity, and specificity was acceptable for all analytes/matrices based on quantitation and confirmation ion transition data; however, ground, surface and drinking water matrices were not characterized. The LOD for the method was also not equivalent in the ECM and ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Pyrifluquinazon	50306301[1] & 49928711 (Appendix 1)[2]	49928711[3]		Ground, Surface, and Drinking Water	24/01/2013 (Final report) 14/02/2013 (Amendment 1)	Nichino America, Inc.	LC/MS/MS	0.50 µg/L (ILV) 0.1 µg/L (ECM)
IV-01								
IV-02								
IV-15								
IV-27								
IV-28								
IV-203								

1 In the ECM, ground water obtained from a borehole in Sudbury, surface water obtained from Diss Mere, Norfolk, and drinking water obtained from a tap in the Huntingdon Life Sciences Environmental Analysis Department were used in the study, but not characterized (p. 23 of MRID 50306301). Water matrices sources were not further specified.

2 Huntingdon Life Sciences Analytical Procedure LMS/0075-01R was provided in Appendix 1 of the ILV, but it was a method only pp. 10, 28; Appendix 1, pp. 90-94 of MRID 49928711. No performance data for Huntingdon Life Sciences Analytical Procedure LMS/0075-01R was provided. No water matrices were specified or characterized.

3 In the ILV, ground water (pH 7.27, total hardness 432 mg/L, DO 7.84 mg/L, dissolved organic carbon ND, total organic carbon 0.13 ppb), surface water (pH 7.52, total hardness 32 mg/L, DO 9.42 mg/L, dissolved organic carbon 2.52 ppb, total organic carbon 3.43 ppb), and drinking water (pH 8.81, total hardness 146 mg/L, DO 9.64 mg/L, dissolved organic carbon ND, total organic carbon 0.95 ppb) matrices were well characterized (p. 17 of MRID 49928711). Water characterization was performed by ABC Laboratories (GLP). Water matrices were collected from Joann Grant, Truxton, Missouri; sources were not further specified.

I. Principle of the Method

ECM [MRID 50306301 & Appendix 1, pp. 90-94 of MRID 49928711 (Method Only)]: Samples (200 mL) of water in a 250 mL separatory funnel were fortified and immediately add 20 mL of methanol and 10 mL of dichloromethane (p. 25 of MRID 50306301; Appendix 1, p. 92 of MRID 49928711). After shaking vigorously for *ca*. 1 minute, the phases were allowed to separate. The lower dichloromethane layer was transferred to a scintillation vial through anhydrous sodium sulphate (*ca*. 2 g, held in a SPE reservoir with glass wool). The water was extracted with 10 mL of dichloromethane. After shaking vigorously for *ca*. 1 minute, the phases were allowed to separate. The lower dichloromethane layer was transferred to the same scintillation vial through the same anhydrous sodium sulphate. The water was extracted with 5 mL of dichloromethane in the same manner as before. The solvent was removed under a stream of nitrogen in a water bath set at 25°C until just dry. The residue was reconstituted in 2 mL of methanol using ultrasonication and vortex mixing prior to LC/MS/MS analysis.

Samples were analyzed for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27 and IV-28 using Micromass Quattro LC [Phenomenex Aqua C_{18}, 2 mm x 50 mm column (pore size 125 Å); column temperature not reported] using a mobile phase of (A) 0.01M ammonium formate in water:methanol:formic acid (90:10:0.1, v:v:v) and (B) methanol:formic acid (100:0.1, v:v) [percent A:B at 0 min. 100:0, 2-5 min. 0:100, 5.5-7.5 min. 100:0] with MS/MS-ESI (electrospray ionization) detection in positive ion mode and multiple reaction monitoring (MRM; pp. 4C, 26 of MRID 50306301; Appendix 1, p. 93 of MRID 49928711). Injection volume was 10 µL. Analytes were identified using two ion transitions; quantitation (Q) and confirmation (C) designations were not provided. Ion transitions monitored were *m/z* 465→107 and *m/z* 465→92 for pyrifluquinazon, *m/z* 423→107 and *m/z* 423→92 for IV-01, *m/z* 421→105 and *m/z* 421→107 for IV-02, *m/z* 437→92 and *m/z* 437→107 for IV-15, *m/z* 453→107 and *m/z* 453→105 for IV-27, and *m/z* 437→105 and *m/z* 437→148 for IV-28. Approximate retention time was 3 minutes for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27 and IV-28.

Samples were analyzed for IV-203 using Waters Acquity TQD [Acquity UPLC BEH C_{18}, 2.1 mm x 50 mm column (1.7 µm particle size; pore size 130 Å); column temperature 45°C] using a mobile phase of (A) water:acetonitrile:acetic acid (90:10:0.1, v:v:v) and (B) acetonitrile:acetic acid (100:0.1, v:v) [percent A:B at 0-0.2 min. 100:0, 2-2.5 min. 5:95, 3-4 min. 100:0] with MS/MS-ESI (electrospray ionization) detection in negative ion mode and multiple reaction monitoring (MRM; pp. 4D, 27 of MRID 50306301; Appendix 1, p. 94 of MRID 49928711). Injection volume was 10 µL. IV-203 was identified using two ion transitions; quantitation (Q) and confirmation (C) designations were not provided. Ion transitions monitored were *m/z* 329→309 and *m/z* 329→289. Approximate retention time for IV-203 was 1.4 minutes.

ILV (MRID 49928711): In the ILV, the ECM method was performed as written, except that the 200 g/mL IV-02 standard was prepared in acetone:methanol (50:50, v:v), the water samples were diluted prior to analysis due to the fact that the LC/MS/MS instrument of the ILV was more sensitive than that used in the ECM, and the injection volume for IV-203 was reduced to 5 µL for drinking and surface water injections (pp. 18-21 of MRID 49928711). Additionally, the controls were not determined to be free of interferences prior to the ILV, but were determined to be free of interferences at the retention times of the target analytes during the course of the study.

The ILV analytical instrument for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 was an Applied Biosystems/Sciex API 5000 MS (Phenomenex Aqua C18 column, 2 mm x 50 mm, 5 µm column; oven temperature 30°C) employing turbo ion spray (TIS) interface. All other analytical parameters were the same as those in the ECM. Approximate retention time was *ca*. 2.6 minutes for pyrifluquinazon and IV-02 and *ca*. 2.5 minutes for IV-01, IV-15, IV-27 and IV-28. The ILV analytical instrument for IV-203 was an Applied Biosystems/Sciex API 5000 MS (Acquity BEH C18 column, 2 mm x 50 mm, 1.7 µm column; oven temperature 45°C) employing TIS interface and injection volume of 5 µL (drinking and surface water injections) and 10 µL (ground water injections). All other analytical parameters were the same as those in the ECM. Approximate retention time for IV-203 was 1.6 minutes. Analytes were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 465.1→107 (Q) and *m/z* 465.1→92 (C) for pyrifluquinazon, *m/z* 423→107 (Q) and *m/z* 423→92 (C) for IV-01, *m/z* 421→105 (Q) and *m/z* 421→107 (C) for IV-02, *m/z* 437→107 (Q) and *m/z* 437→92 (C) for IV-15, *m/z* 453→107 (Q) and *m/z* 453→105 (C) for IV-27, *m/z* 437→105 (Q) and *m/z* 437→148 (C) for IV-28, and *m/z* 329→309 (Q) and *m/z* 329→289 (C) for IV-203.

LOD/LOQ

In the ECM (MRID 50306301 & Appendix 1, pp. 90-94 of MRID 49928711), the LOQ and LOD for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 in water matrices were reported as 0.1 µg/L and 0.025 µg/L (2.5 ng/mL), respectively (pp. 4C-4D, 11, 15 of MRID 50306301; Appendix 1, pp. 93-94 of MRID 49928711).

In the ILV (MRID 49928711), the LOQ and LOD for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 in water matrices were reported as 0.50 µg/L (pp. 10, 13 of MRID 49928711). The LOD was reported as 0.15 µg/L.

II. Recovery Findings

ECM (MRID 50306301): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of pyrifluquinazon and its metabolites IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 in ground, surface and drinking water at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ; pp. 31-34; Tables 8-49, pp. 41-82). Pyrifluquinazon and its metabolites were identified via LC/MS/MS using two ion transitions; performance data from the primary and confirmatory methods was comparable. The ground water obtained from a borehole in Sudbury, surface water obtained from Diss Mere, Norfolk, and drinking water obtained from a tap in the Huntingdon Life Sciences Environmental Analysis Department were used in the study, but not characterized (p. 23 of MRID 50306301). Water matrices sources were not further specified.

ILV (MRID 49928711): Mean recoveries and RSDs were within guideline requirements for analysis of pyrifluquinazon and metabolites IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 in ground, surface and drinking water at fortification levels of 0.5 µg/L (LOQ) and 5.0 µg/L (10×LOQ), except for IV-203 in surface water (mean, 68% LOQ and 66% 10×LOQ) and drinking water (mean, 68% LOQ and 69% 10×LOQ; pp. 11-12, 25-26 and Tables 1-3, pp. 29-34). Pyrifluquinazon and its metabolites were identified via LC/MS/MS using two ion transitions; however, recovery results were only reported for the quantitation ion transition. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The ground water (pH 7.27, total hardness 432 mg/L, DO 7.84 mg/L, dissolved organic carbon ND, total organic carbon 0.13 ppb), surface water (pH 7.52, total hardness 32 mg/L, DO 9.42 mg/L, dissolved organic carbon 2.52 ppb, total organic carbon 3.43 ppb), and drinking water (pH 8.81, total hardness 146 mg/L, DO 9.64 mg/L, dissolved organic carbon ND, total organic carbon 0.95 ppb) matrices were well characterized (p. 17). Water characterization was performed by ABC Laboratories (GLP). Water matrices were collected from Joann Grant, Truxton, Missouri; sources were not further specified. The ILV validated the method in the first trial with insignificant modifications to standard preparation and analytical procedure (pp. 10, 19-21).

Table 2. Initial Validation Method Recoveries for Pyrifluquinazon and Its Metabolites IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground Water					
	Quantitation ion transition					
Pyrifluquinazon	0.1 (LOQ)	5	84-101	95	7.4	7.9
	1.0	5	92-113	106	8.1	7.7
IV-01	0.1 (LOQ)	5	89-104	95	6.5	6.8
	1.0	5	96-113	103	6.2	6.0
IV-02	0.1 (LOQ)	5	93-105	100	4.7	4.7
	1.0	5	95-105	100	4.3	4.3
IV-15	0.1 (LOQ)	5	90-111	102	8.2	8.0
	1.0	5	96-119	108	8.9	8.2
IV-27	0.1 (LOQ)	5	78-101	89	9.7	10.9
	1.0	5	86-113	98	10.9	11.1
IV-28	0.1 (LOQ)	5	83-95	87	5.2	6.0
	1.0	5	90-101	97	4.4	4.5
IV-203	0.1 (LOQ)	5	75-81	79	2.4	3.1
	1.0	5	81-84	82	1.3	1.6
	Confirmation ion transition					
Pyrifluquinazon	0.1 (LOQ)	5	88-107	96	7.1	7.4
	1.0	5	80-95	87	6.5	7.4
IV-01	0.1 (LOQ)	5	95-114	107	8.6	8.0
	1.0	5	95-112	102	7.2	7.0
IV-02	0.1 (LOQ)	5	86-99	92	4.9	5.3
	1.0	5	100-110	105	4.3	4.1
IV-15	0.1 (LOQ)	5	91-108	100	8.3	8.3
	1.0	5	95-123	106	12.2	11.5
IV-27	0.1 (LOQ)	5	96-120	105	9.4	8.9
	1.0	5	72-105	87	12.3	14.1
IV-28	0.1 (LOQ)	5	97-118	106	8.4	7.9
	1.0	5	81-95	88	5.9	6.7
IV-203	0.1 (LOQ)	5	70-79	75	3.8	5.2
	1.0	5	83-91	86	3.5	4.0
	Surface Water					
	Quantitation ion transition					
Pyrifluquinazon	0.1 (LOQ)	5	102-112	106	4.3	4.1
	1.0	5	91-107	99	6.6	6.7
IV-01	0.1 (LOQ)	5	85-113	95	11.0	11.5
	1.0	5	95-115	105	8.6	8.2
IV-02	0.1 (LOQ)	5	88-102	98	5.5	5.7
	1.0	5	91-101	94	4.3	4.6

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IV-15	0.1 (LOQ)	5	94-104	99	4.1	4.2
	1.0	5	105-129	115	9.6	8.4
IV-27	0.1 (LOQ)	5	91-113	103	9.5	9.2
	1.0	5	100-107	103	2.9	2.8
IV-28	0.1 (LOQ)	5	70-84	77	6.4	8.3
	1.0	5	92-101	97	4.0	4.1
IV-203	0.1 (LOQ)	5	69-79	74	3.8	5.2
	1.0	5	77-83	79	2.3	2.9
	Confirmation ion transition					
Pyrifluquinazon	0.1 (LOQ)	5	91-108	99	6.8	6.9
	1.0	5	79-110	91	11.9	13.1
IV-01	0.1 (LOQ)	5	75-103	92	10.9	11.8
	1.0	5	93-111	103	7.0	6.8
IV-02	0.1 (LOQ)	5	79-92	84	4.8	5.8
	1.0	5	90-101	94	4.3	4.6
IV-15	0.1 (LOQ)	5	81-113	93	12.2	13.1
	1.0	5	94-106	97	5.2	5.4
IV-27	0.1 (LOQ)	5	95-120	107	9.1	8.5
	1.0	5	84-115	92	13.1	14.3
IV-28	0.1 (LOQ)	5	75-110	91	12.9	14.1
	1.0	5	83-115	95	12.3	13.0
IV-203	0.1 (LOQ)	5	65-86	77	8.6	11.2
	1.0	5	79-88	84	3.5	4.2
	Drinking Water					
	Quantitation ion transition					
Pyrifluquinazon	0.1 (LOQ)	5	83-90	86	3.2	3.8
	1.0	5	94-105	98	5.1	5.3
IV-01	0.1 (LOQ)	5	83-96	90	5.7	6.3
	1.0	5	81-103	88	8.8	10.0
IV-02	0.1 (LOQ)	5	97-113	105	6.7	6.3
	1.0	5	107-114	111	2.5	2.3
IV-15	0.1 (LOQ)	5	94-112	105	7.6	7.3
	1.0	5	82-119	103	13.9	13.5
IV-27	0.1 (LOQ)	5	70-92	79	10.7	13.4
	1.0	5	83-119	96	13.8	14.4
IV-28	0.1 (LOQ)	5	73-91	82	7.6	9.2
	1.0	5	87-106	94	7.4	7.9
IV-203	0.1 (LOQ)	5	68-75	71	2.8	3.9
	1.0	5	75-83	80	3.4	4.3

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Confirmation ion transition					
Pyrifluquinazon	0.1 (LOQ)	5	90-12	101	11.4	11.3
	1.0	5	75-105	87	12.5	14.3
IV-01	0.1 (LOQ)	5	82-104	95	8.4	8.9
	1.0	5	78-101	89	8.9	10.0
IV-02	0.1 (LOQ)	5	72-90	80	9.3	11.6
	1.0	5	101-123	110	8.1	7.3
IV-15	0.1 (LOQ)	5	75-105	85	12.1	14.2
	1.0	5	98-125	111	11.5	10.3
IV-27	0.1 (LOQ)	5	102-108	106	2.7	2.5
	1.0	5	79-102	93	9.1	9.8
IV-28	0.1 (LOQ)	5	92-117	106	10.8	10.2
	1.0	5	75-103	89	11.5	13.0
IV-203	0.1 (LOQ)	5	68-82	73	5.5	7.5
	1.0	5	84-92	86	3.5	4.0

Data (uncorrected recovery results, pp. 28-29 of MRID 50306301) were obtained from pp. 31-34; Tables 8-49, pp. 41-82 of MRID 50306301.

1 The ground water obtained from a borehole in Sudbury, surface water obtained from Diss Mere, Norfolk, and drinking water obtained from a tap in the Huntingdon Life Sciences Environmental Analysis Department were used in the study, but not characterized (p. 23 of MRID 50306301). Water matrices sources were not further specified.

2 Ion transitions monitored were as follows [quantitation (Q) and one for confirmation (C)]: *m/z* 465.1→107 (Q) and *m/z* 465.1→92 (C) for pyrifluquinazon, *m/z* 423→107 (Q) and *m/z* 423→92 (C) for IV-01, *m/z* 421→105 (Q) and *m/z* 421→107 (C) for IV-02, *m/z* 437→107 (Q) and *m/z* 437→92 (C) for IV-15, *m/z* 453→107 (Q) and *m/z* 453→105 (C) for IV-27, *m/z* 437→105 (Q) and *m/z* 437→148 (C) for IV-28, and *m/z* 329→309 (Q) and *m/z* 329→289 (C) for IV-203 (pp. 4C-4D, 26-27).

Table 3. Independent Validation Method Recoveries for Pyrifluquinazon and Its Metabolites IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 in Water[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground Water					
	Quantitation ion[4]					
Pyrifluquinazon	0.5 (LOQ)	5	72-86	80	5.29	6.62
	5.0	5	84-97	92	4.80	5.22
IV-01	0.5 (LOQ)	5	81-93	88	4.63	5.26
	5.0	5	92-103	100	4.58	4.59
IV-02	0.5 (LOQ)	5	76-102	89	9.40	10.56
	5.0	5	89-101	99	5.47	5.54
IV-15	0.5 (LOQ)	5	73-90	81	6.99	8.59
	5.0	5	87-100	96	5.40	5.64
IV-27	0.5 (LOQ)	5	72-81	77	3.42	4.46
	5.0	5	72-96	86	8.79	10.18
IV-28	0.5 (LOQ)	5	68-84	78	6.13	7.84
	5.0	5	80-98	91	6.87	7.53
IV-203	0.5 (LOQ)	5	72-79	75	2.66	3.54
	5.0	5	73-85	79	4.20	5.32
	Surface Water					
	Quantitation ion[4]					
Pyrifluquinazon	0.5 (LOQ)	5	80-89	84	3.42	4.08
	5.0	5	88-94	89	2.52	2.82
IV-01	0.5 (LOQ)	5	80-90	86	4.40	5.11
	5.0	5	89-97	92	3.27	3.54
IV-02	0.5 (LOQ)	5	73-88	81	5.64	6.94
	5.0	5	93-97	95	1.70	1.79
IV-15	0.5 (LOQ)	5	80-96	87	6.88	7.89
	5.0	5	82-93	88	4.42	5.01
IV-27	0.5 (LOQ)	5	73-81	78	3.12	3.99
	5.0	5	80-91	85	4.03	4.73
IV-28	0.5 (LOQ)	5	72-83	76	5.05	6.65
	5.0	5	79-86	82	3.25	3.95
IV-203	0.5 (LOQ)	5	61-75	**68**	5.16	7.55
	5.0	5	60-68	**66**	3.23	4.92
	Drinking Water					
	Quantitation ion[4]					
Pyrifluquinazon	0.5 (LOQ)	5	87-92	89	1.90	2.13
	5.0	5	78-97	89	7.96	8.93
IV-01	0.5 (LOQ)	5	88-96	91	2.88	3.17
	5.0	5	76-103	91	10.03	11.07

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IV-02	0.5 (LOQ)	5	84-94	90	3.81	4.25
	5.0	5	75-100	90	9.20	10.23
IV-15	0.5 (LOQ)	5	83-90	86	2.72	3.16
	5.0	5	73-100	89	10.08	11.35
IV-27	0.5 (LOQ)	5	84-91	88	2.91	3.29
	5.0	5	74-101	90	9.66	10.79
IV-28	0.5 (LOQ)	5	75-87	81	4.92	6.10
	5.0	5	68-91	85	10.25	12.05
IV-203	0.5 (LOQ)	5	61-74	**68**	5.77	8.54
	5.0	5	61-76	**69**	5.72	8.26

Data (uncorrected recovery results, pp. 22-23) were obtained from pp. 11-12, 25-26 and Tables 1-3, pp. 29-34 of MRID 49928711.

1 The ground water (pH 7.27, total hardness 432 mg/L, DO 7.84 mg/L, dissolved organic carbon ND, total organic carbon 0.13 ppb), surface water (pH 7.52, total hardness 32 mg/L, DO 9.42 mg/L, dissolved organic carbon 2.52 ppb, total organic carbon 3.43 ppb), and drinking water (pH 8.81, total hardness 146 mg/L, DO 9.64 mg/L, dissolved organic carbon ND, total organic carbon 0.95 ppb) matrices were well characterized (p. 17 of MRID 49928711). Water characterization was performed by ABC Laboratories (GLP). Water matrices were collected from Joann Grant, Truxton, Missouri; sources were not further specified.

2 Ion transitions monitored were as follows [quantitation (Q) and one for confirmation (C)]: *m/z* 465.1→107 (Q) and *m/z* 465.1→92 (C) for pyrifluquinazon, *m/z* 423→107 (Q) and *m/z* 423→92 (C) for IV-01, *m/z* 421→105 (Q) and *m/z* 421→107 (C) for IV-02, *m/z* 437→107 (Q) and *m/z* 437→92 (C) for IV-15, *m/z* 453→107 (Q) and *m/z* 453→105 (C) for IV-27, *m/z* 437→105 (Q) and *m/z* 437→148 (C) for IV-28, and *m/z* 329→309 (Q) and *m/z* 329→289 (C) for IV-203 (p. 21).

3 Results were reported from the first trial (p. 10).

4 Recovery results were only reported for the quantitation ion transition. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

III. Method Characteristics

In the ECM (MRID 50306301; Appendix 1, pp. 90-94 of MRID 49928711), the LOQ and LOD for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 in water matrices were reported as 0.1 µg/L and 0.025 µg/L (2.5 ng/mL), respectively (pp. 4C-4D, 11, 15, 34 of MRID 50306301; Appendix 1, pp. 93-94 of MRID 49928711). The LOQ was defined as the lowest fortification level at which acceptable recovery data was obtained. No calculations or comparison to background noise were reported for the LOQ. No justification for LOD was reported.

In the ILV (MRID 49928711), the LOQ and LOD for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 in water matrices were reported as 0.50 µg/L (pp. 10, 13 of MRID 49928711). The LOD was reported as 0.15 µg/L. No justification for the LOQ and LOD was reported.

Table 4. Method Characteristics

Analyte		Pyrifluquinazon	IV-01	IV-02	IV-15	IV-27	IV-28	IV-203
Limit of Quantitation (LOQ)	ECM	0.1 µg/L						
	ILV	0.50 µg/L						
Limit of Detection (LOD)	ECM	0.025 µg/L (2.5 ng/mL)						
	ILV	0.15 µg/L						
Linearity (calibration curve r^2 and concentration range)	ECM	r^2 = 0.9958 (Q) r^2 = 0.9975 (C)	r^2 = 0.9945 (Q) r^2 = 0.9973 (C)	r^2 = 0.9987 (Q) r^2 = 0.9994 (C)	r^2 = 0.9998 (Q) r^2 = 0.9955 (C)	r^2 = 0.9990 (Q) r^2 = 0.9953 (C)	r^2 = 0.9979 (Q) r^2 = 0.9968 (C)	r^2 = 0.9997 (Q)[1] r^2 = 0.9988 (C)[1]
		(2.5-250 ng/mL or 0.025-2.5 µg/L in matrix)						
	ILV[2,3]	r^2 = **0.9948** (Q)	r^2 = 0.9974 (Q)	r^2 = 0.9966 (Q)	r^2 = 0.9984 (Q)	r^2 = 0.9976 (Q)	r^2 = 0.9956 (Q)	r^2 = 0.9988 (Q)
		(0.1-2.5 ng/mL)					(0.25-2.5 ng/mL)	(0.1-2.5 ng/mL)
Repeatable	ECM[4]	Yes at LOQ and 10×LOQ						
	ILV[2,5,6]	Yes at LOQ and 10×LOQ						Yes at LOQ and 10×LOQ in ground water; **No** at the LOQ and 10×LOQ in surface and drinking water
Reproducible		**Could not be determined – ILV LOQ ≠ ECM LOQ**						
Specific	ECM	Yes, no matrix interferences were noted. More baseline noise was noted in the C chromatograms than the Q chromatograms.						
	ILV	Minor baseline noise was observed. Only quantitation ion transition chromatograms were provided.[2]						
		Yes, no matrix interferences were noted.		Yes, matrix interferences in ground water were <5% of the LOQ (based on peak area). Yes, no matrix interferences were noted in surface and drinking water.	Yes, no matrix interferences were noted.		Yes, matrix interferences in ground water were <10% of the LOQ (based on peak area). Yes, no matrix interferences were noted in surface and drinking water;	Yes, matrix interferences in drinking water were <5% of the LOQ (based on peak area). Yes, no matrix interferences were noted in ground and surface water

Analyte		Pyrifluquinazon	IV-01	IV-02	IV-15	IV-27	IV-28	IV-203
							however, major baseline noise was significant near the analyte peak.[7]	

Data were obtained from pp. 4C-4D, 11, 15, 34 (LOD/LOQ); pp. 31-34; Tables 8-49, pp. 41-82 (recovery data); Tables 1-7, pp. 37-40 (calibration data); Figures 1-7, pp. 84-90 (calibration curves); Figures 15-35, pp. 98-118 (chromatograms) of MRID 50306301; pp. 11-12, 25-26; Tables 1-3, pp. 29-34 (recovery data); Figure 4-10, pp. 47-53 (calibration curves); Figures 11-19, pp. 54-89 (chromatograms) of MRID 49928711 (ILV); DER Attachment 2. Q = quantitation ion.

1 Quadratic equation used instead of linear regression.

2 Two ion transitions were monitored for each analyte; however, recovery results and chromatograms were only reported for the quantitation ion transition. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

3 Calibration coefficients (r^2) were reviewer-calculated from r values provided in the study report (DER Attachment 2).

4 In the ECM, ground water obtained from a borehole in Sudbury, surface water obtained from Diss Mere, Norfolk, and drinking water obtained from a tap in the Huntingdon Life Sciences Environmental Analysis Department were used in the study, but not characterized (p. 23 of MRID 50306301). Water matrices sources were not further specified.

5 In the ILV, ground water (pH 7.27, total hardness 432 mg/L, DO 7.84 mg/L, dissolved organic carbon ND, total organic carbon 0.13 ppb), surface water (pH 7.52, total hardness 32 mg/L, DO 9.42 mg/L, dissolved organic carbon 2.52 ppb, total organic carbon 3.43 ppb), and drinking water (pH 8.81, total hardness 146 mg/L, DO 9.64 mg/L, dissolved organic carbon ND, total organic carbon 0.95 ppb) matrices were well characterized (p. 17 of MRID 49928711). Water characterization was performed by ABC Laboratories (GLP). Water matrices were collected from Joann Grant, Truxton, Missouri; sources were not further specified.

6 Results were reported from the first trial with insignificant modifications to standard preparation and analytical procedure (pp. 10, 19-21 of MRID 49928711).

7 Based on Figures 14, p. 68 and Figure 15, pp. 72, 76 of MRID 49928711.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. In the file name of the ECM MRID 50306301, it was noted that the ECM was an updated version of MRID 49083753. CDM/CSS-Dynamac JV was not provided with MRID 49083753.

2. An ECM/ILV method validation set was previously submitted and reviewed for pyrifluquinazon and its metabolites in water; however, the ECM which was validated by ILV MRID 49928711 was only provided in the Appendix of the ILV as a method only (pp. 10, 28; Appendix 1, pp. 90-94 of MRID 49928711). The MRID 48306934 was submitted as the ECM for ILV MRID 49928711; however, the methods were not the same. The DER for MRIDs 48306934 & 49928711 was written by CDM Smith/ CSS-Dynamac JV Primary Reviewer Lisa Muto, with CDM Smith/ CSS-Dynamac JV Secondary Reviewer Kathleen Ferguson. The reviewer determined that an updated, complete ECM/ILV method validation set should be submitted for pyrifluquinazon or pyrifluquinazon and its metabolites in water.

   For this review, ECM data was replaced, and ILV was verified and edited, as needed.

3. The reproducibility of the ECM method could not be determined since the validated LOQ of the ECM (0.1 µg/L; pp. 4C-4D, 11, 15, 34 of MRID 50306301) was not equivalent to the validated LOQ of the ILV (0.50 µg/L; pp. 10, 13 of MRID 49928711). Also, no ILV samples were prepared at the validated LOQ of the ECM (0.1 µg/L). No calculations or comparison to background noise were reported for the LOQ, so the reason for the LOQ difference could not be determined. The ILV did not note the LOQ difference in the Protocol Amendments and Deviations (Appendix 5, p. 148 of MRID 49928711).

4. The following deficiencies were noted in the ILV MRID 49928711:

   Mean recoveries were not within guideline requirements for analysis of IV-203 in surface water (mean, 68% LOQ and 66% 10×LOQ) and drinking water (mean, 68% LOQ and 69% 10×LOQ; pp. 11-12, 25-26 and Tables 1-3, pp. 29-34 of MRID 49928711). The study author noted that "these recovery results were accepted by the Sponsor as IV-203 is found at very low concentrations in the environment and possesses very low mammalian acute toxicity" (p. 27). In the communications, the Sponsor also noted that IV-203 is a terminal metabolite which is a soil metabolite only present at concentrations of 0.01-0.03 ppm (Appendix 4, p. 145). The Sponsor also noted that the average recovery for IV-203 is 69.67% which could be rounded up to 70%.

   In the representative chromatograms for IV-28 in surface and drinking water, major baseline noise was noted near the analyte peak (Figures 14, p. 68; Figure 15, pp. 72, 76 of MRID 49928711). Some interference in the integration of the LOQ peak was noted in these chromatograms.

   The linearity of the pyrifluquinazon calibration curve was unsatisfactory (r^2 <0.995): $r^2 = 0.9948$ (Figure 4, p. 47 of MRID 49928711; DER Attachment 2).

Two ion transitions were monitored for each analyte; however, recovery results and chromatograms were only reported for the quantitation ion transition. While the reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method, the ILV is typically expected to be as comprehensive as the ECM and a validation of all aspects of the ECM. Additionally, the reviewer noted that this deviation from the ECM was not reported in the ILV (p. 19 of MRID 49928711).

5. In the ECM (MRID 50306301), the water matrices were not characterized, and water sources were not specified (p. 23 of MRID 50306301).

6. The reviewer noted that the IV-203 calibration data was evaluated using a quadratic equation instead of linear regression (Tables 1-7, pp. 37-40; Figures 1-7, pp. 84-90 of MRID 50306301).

7. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 pp. 4C-4D, 11, 15, 34 of MRID 50306301; Appendix 1, pp. 93-94 of MRID 49928711; pp. 10, 13 of MRID 49928711). No calculations or comparisons to noise level were reported. In the ECM, LOQ was defined as the lowest fortification level at which acceptable recovery data was obtained. No calculations or comparison to background noise were reported for the LOQ. No justification for LOD was reported. In the ILV, no justification for the LOQ and LOD was reported. The LOQ and LOD of the ILV differed from those of the ECM.

8. In the ILV, communications between the ILV and Sponsor Representative (Ken Chisholm of Nichino) regarding the ECM were summarized and detailed in the study (pp. 10, 24, 27; Appendix 4, pp. 131-147 of MRID 49928711). Communication regarded the approval of equivalent techniques, correction of typographical errors, clarification of some technical aspects of the method, and recovery updates between the Sponsor Representative and Study Director. The ILV was provided with the additional details about the analytical columns which was included in the ECM Amendment. The ILV discussed the low recovery of IV-203 with the Study Sponsor. No direct contact or technical guidance was provided to the ILV personnel by the ECM personnel.

9. The reviewer noted that the purity of the IV-27 standard was >90%, but significantly lower than the other standards, 91.5% (p. 21 of MRID 50306301; p. 16 of MRID 49928711).

10. In the ILV, the matrix effects of the water samples were evaluated to determine the water control suitability; only control samples free of any interferences in the area of analyte elution (corresponding to analyte residue levels of <30% of the LOQ) were chosen for use in the study (pp. 23-24 of MRID 49928711).

11. In the ECM, the sample extract stability for all analytes/matrices was determined to be up to 5 days when stored at *ca*. -20°C in the dark (pp. 15, 34; Table 50, p. 83 of MRID 50306301).

12. It was reported for the ILV that one batch of thirteen samples required one workday (8 hours) with LC/MS/MS performed overnight (pp. 13, 27 of MRID 49928711).

13. The MV was previously submitted with EPA MRID No.: 48306934. Kendall, T.Z., W.B. Nixon. 2009. ANALYTICAL METHOD VERIFICATION FOR THE DETERMINATION OF PYRIFLUQUINAZON IN FRESHWATER AND SALTWATER. Wildlife International, Ltd. Project No.: 397C-106. Report prepared by Wildlife International, Ltd., Easton, Maryland, sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 52 pages. Final report issued June 11, 2009. The LOQ for the ECM MRID 48306934 method in freshwater was equal to the LOQ of the ILV MRID 49928711, but this ECM method was not performed in the ILV (see previously submitted DER for MRIDs 48306934/49928711). In ECM MRID 48306934 for freshwater and saltwater, no samples were prepared at the LOQ or 10×LOQ, and the number of samples was insufficient for all analyses, n = 3. The LOD for the method was not reported.

V. References

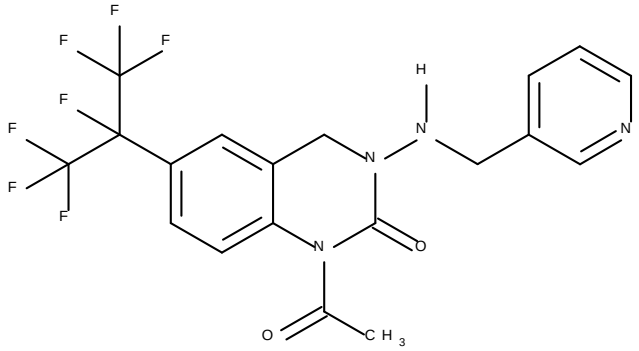
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

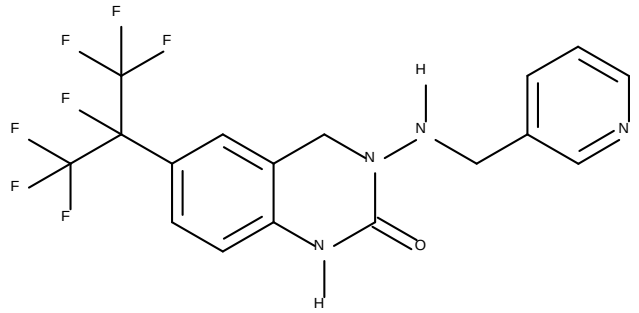
Pyrifluquinazon (NNI-0101)

IUPAC Name: 1-Acetyl-1,2,3,4-tetrahydro-3-[(3-pyridylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]quinazolin-2-one
CAS Name: 1-Acetyl-3,4-dihydro-3-[(3-pyridinylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-2(1H)-quinazolinone
CAS Number: 337458-27-2
SMILES String: [H]N(Cc1cccnc1)N2Cc3cc(ccc3N(C2=O)C(=O)C)C(C(F)(F)F)(C(F)(F)F)F



IV-01 (NNI-0101-1H)

IUPAC Name: 3-[(Pyridin-3-ylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-3,4-dihydro-1H-quinazolin-2-one
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H]N1c2ccc(cc2CN(C1=O)N([H])Cc3cccnc3)C(C(F)(F)F)(C(F)(F)F)F



IV-02 (NNI-0101-1H-imino)

IUPAC Name:	3-[(Pyridin-3-ylmethylene)amino]-6-[l,2,2,2-tetrafluoro-l-(trifluoromethyl)ethyl]-3,4-dihydro-1H-quinazolin-2-one
CAS Name:	Not reported
CAS Number:	Not reported
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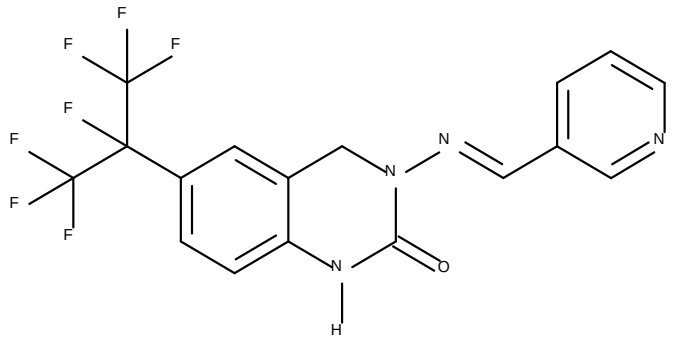
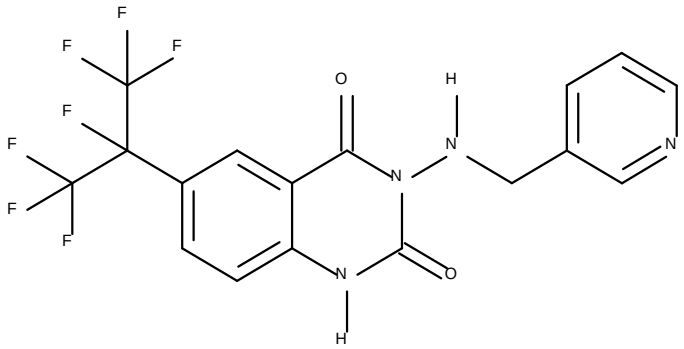


IV-15 (NNI-0101-1H-4-oxo)

IUPAC Name:	3-[(Pyridin-3-ylmethyl)amino]-6-[l,2,2,2-tetrafluoro-l-(trifluoromethyl)ethyl]-1H-quinazolin-2,4-dione
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	[H]n1c2ccc(cc2c(=O)n(c1=O)N([H])Cc3cccnc3)C(C(F)(F)F)(C(F)(F)F)F



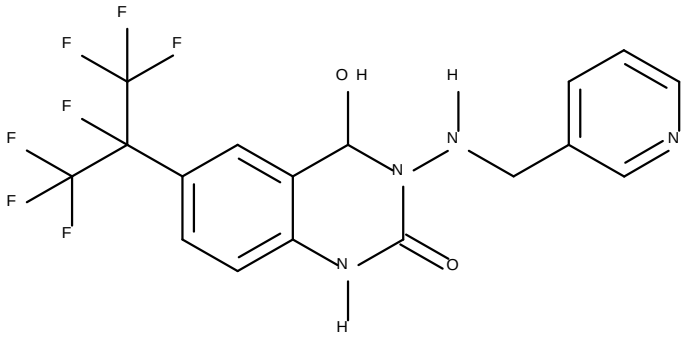
IV-27 (NNI-0101-1H-4-OH)

IUPAC Name: l,2,3,4-Tetrahydro-4-hydroxy-3-[(3-pyridylmethyl)amino]-6-[l,2,2,2-tetrafluoro-l-(trifluoromethyl)ethyl]quinazolin-2-one

CAS Name: Not reported

CAS Number: Not reported

SMILES String: [H]N1c2ccc(cc2C(N(C1=O)N([H])Cc3cccnc3)O)C(C(F)(F)F)(C(F)(F)F)F



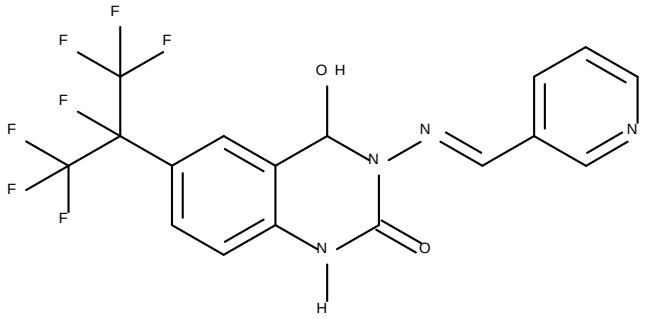
IV-28 (NNI-0101-1H-imino-4-OH)

IUPAC Name: 4-Hydroxy-3-[(3-pyridin-3-ylmethylene)amino]-6-[l,2,2,2-tetrafluoro-l-(trifluoromethyl)ethyl]-3,4-dihydro-lH-quinazolin-2-one

CAS Name: Not reported

CAS Number: Not reported

SMILES String: [H]N1c2ccc(cc2C(N(C1=O)/N=C/c3cccnc3)O)C(C(F)(F)F)(C(F)(F)F)F



IV-203 (NNI-0101-quinazolinedione)

IUPAC Name:	6-[1,2,2,2-Tetrafluoro-1-trifluoromethyl)ethyl]-1H-quinazolin-2,4-dione
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	[H]n1c2ccc(cc2c(=O)n(c1=O)[H])C(C(F)(F)F)(C(F)(F)F)F